STRATEGIC DIAGNOSTICS INC.
EnviroGard® 2,4-D Test Kit
72900

Intended Use
The EnviroGard 2,4-D Test Kit is a qualitative ("yes/no") or semi-quantitative test for the detection of 2,4-Dichlorophenoxyacetic acid (2,4-D) and closely related residues in water or other aqueous solutions. The test kit can be used in the laboratory or on-site without any special training. With the EnviroGard 2,4-D Test Kit, you can read results visually or perform a more precise analysis with a photometer.

Test Principles
The EnviroGard 2,4-D test is based on the use of polyclonal antibodies which bind either 2,4-D residues or 2,4-D-enzyme conjugate. The 2,4-D in the sample competes with 2,4-D-enzyme conjugate for a limited number of antibody binding sites. Antibodies that bind 2,4-D are immobilized to the walls of the test tubes.

Since there are the same number of antibody binding sites on every test tube, and each test tube receives the same number of 2,4-D-enzyme conjugate molecules, a sample which contains a low concentration of 2,4-D enables the antibody to bind many 2,4-D-enzyme conjugate molecules. Therefore, a low concentration of 2,4-D will produce a dark blue solution. Conversely, a high concentration of 2,4-D will allow fewer 2,4-D-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to 2,4-D concentration.
Darker color = lower concentration.
Lighter color = higher concentration.

Performance Characteristics
The EnviroGard 2,4-D Test Kit will not differentiate between 2,4-D and closely related compounds, but will detect their presence to differing degrees. The following chart shows the approximate lower limit of detection, or Least Detectable Dose (LDD), for the cross-reacting compounds. Concentration is in parts per billion (ppb).

Compound	LDD @ 85% Bo
2,4-D acid	1.4
2,4-D butyl ester	0.2
2,4-D methyl ester	0.6
2,4-D isopropyl ester	4.4
2,4-DB acid	29
2,4-DB butyl ester	65
2,4-DB isobutyl ester	26
2,4-Dichlorophenol	650
2,4,5-T acid	81
2,4,5-TP (Silvex)	>2,600
Dichloroprop	760
MCPA	12
MCPP (Mecoprop)	780
Diclofop	1,500

Precautions
• Store all test kit components at 4°C to 8°C (39°F to 46°F) when not in use.
• Do not freeze test kit components or expose them to temperatures greater than 37°C (99°F).
• Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before beginning the test.
• Do not use test kit components after the expiration date.
• Do not use reagents or test tubes from one test kit with reagents or test tubes from a different test kit.
• Due to the rapid kinetics of the EnviroGard 2,4-D Test Kit, do not analyze more than six test tubes at one time.
• Use approved methodologies to confirm any positive results.
• Do not dilute or adulterate test reagents or use samples not called for in the test procedure. This may give inaccurate results.
• Some solutes and particulates found in untreated ground or surface waters may affect the sensitivity level of this test kit.
• If you are testing something other than water, use a calibrator which has a matrix comparable to your sample.

Before You Start
This test kit contains the following items:

20	antibody-coated test tubes
1	vial of Negative Control
1	vial of 2.0 ppb 2,4-D Calibrator
1	vial of 50 ppb 2,4-D Calibrator
1	vial of 2,4-D Enzyme Conjugate
1	vial of Substrate
1	vial of Chromogen
1	vial of Stop Solution
1	test tube rack

You will also need several other items:

• marking pen for test tubes
• disposable-tip pipette which will measure 160 microliters (µL)
• stopwatch or wristwatch with second hand
• tap or distilled water for washing test tubes
• pipette which will measure 0.5 milliliters (mL) (for photometric interpretation only)
• calculator (optional)
• spectrophotometer (optional)

Perform The Test
1. Remove the test tubes from the plastic bag and mark them as follows:

Tube Marking	Tube Contents
"-"	negative control
"+"	2.0 or 50 ppb calibrator
S1	sample 1
S2	sample 2
S3	sample 3

2. Add 4 drops (160 µL) of Negative Control to the "-" test tube.
3. Add 4 drops (160 µL) of the appropriate calibrator to the "+" test tube.

NOTE: Both calibrators can be used to approximate the concentration levels of your samples to a more accurate degree. However, you should not use more than six test tubes in one test. If you use both calibrators, label them accordingly ("2.0 ppb" and "50 ppb").

4. Add 160 µL of each sample to the corresponding test tube(s). Immediately add 4 drops of 2,4-D-Enzyme Conjugate to each test tube. Gently swirl the test tubes to mix for 2 to 3 seconds.
5. Leave the test tubes undisturbed for 10 minutes, then shake out their contents.
6. Fill the test tubes to overflowing with tap or distilled water, then decant and vigorously shake out the remaining water.

NOTE: Repeat this wash step three more times, being certain to shake out as much water as possible on each wash.

7. Add 4 drops of Substrate to each test tube; follow immediately with 4 drops of Chromogen to each test tube. DO NOT REVERSE THIS ORDER. Gently mix the test tubes for a few seconds.

Interpret The Results

You can either interpret the results visually within 10 minutes after adding the substrate and chromogen to each test tube, or perform a more precise analysis with a photometer.

NOTE: If a blue color does not develop in the negative control test tube within 10 minutes after adding the substrate and chromogen, the test is invalid and must be repeated.

Visual Interpretation

1. Compare the sample test tube to the negative control test tube against a white background. The test tube rack in the kit is well-suited for this purpose.
2. If the sample test tube contains less color than the negative control, the original sample contains 2,4-D, or a cross-reactant (or combination of the same) at a concentration greater than or equal to those levels indicated in the LDD table (see "Performance Characteristics").
3. If you are unable to distinguish a difference between the sample and negative control, there may be pesticides present in the sample but at a very low concentration, near the LDD listed. Use a photometer for a more precise analysis.
4. If a sample test tube contains more color than a calibrator, the original sample contains a lower concentration than that calibrator. Conversely, if a sample test tube contains less color than a calibrator, the sample contains a greater concentration than the calibrator.

Photometric Interpretation

After 10 minutes, add 1 drop (40 µL) of Stop Solution to each test tube and mix well. This will turn the solution yellow.

WARNING: Stop solution is 2.5 N sulfuric acid.

Proceed to the set of instructions suited to your photometer type.

Conventional Spectrophotometers

1. Add 0.5 mL of water to each test tube and gently mix.
2. Adjust the wavelength of your photometer to 450 nanometers (nm) and zero against a water blank.
3. Transfer the reaction liquid to an appropriate cuvette or aspirate it directly into the photometer (depending on the photometer type).
4. Measure and record the absorbance [optical density (OD)] of the negative control, each sample, and the calibrator.
5. Compare the OD of each sample to the OD of the calibrator(s). If the OD of the sample is less than the OD of a calibrator, the sample contains a concentration greater than the concentration of that calibrator. Conversely, if the OD of the sample is greater than the OD of a calibrator, the sample contains a concentration that is lower than the concentration found in that calibrator.

SDI Differential Photometer

1. Add 0.5 mL of water to each test tube and gently mix.
2. Place a test tube containing water in the left (reference) well.
3. Place the negative control test tube into the right (sample) well. Record the OD of the negative control.
4. Remove the negative control test tube and replace it with the next test tube (calibrator or sample) to reactivate the photometer. Record the result. Repeat this procedure to determine the OD for each of the remaining samples.
5. Compare the OD of each sample to the OD of the calibrator(s). If the OD of the sample is less than the OD of a calibrator, the sample contains a concentration greater than the concentration of that calibrator. Conversely, if the OD of the sample is greater than the OD of a calibrator, the sample contains a concentration that is lower than the concentration found in that calibrator.

Ordering Information

Description	Catalog Number
EnviroGard 2,4-D Test Kit	72900

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General Limited Warranty

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